HISTOLOGIC EXAMINATIONS OF FUNGOUS INFECTIONS OF THE NAILS*

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In the study of fungous infections of the nail the histologic examination has been almost entirely neglected by dermatologists. While other nail conditions frequently are examined histologically, investigation of cases of onychomycosis has been confined largely to the microscopic examination of the nail substance, softened in potassium hydroxide. The recognition of fungi in potassium hydroxide preparations is relatively easy and, for this reason, it appears to be unnecessary to look at the histologic picture of the fungous infected nail. Direct microscopic examinations of the nail material in potassium hydroxide, however, yield no certain proof that the fungus exists in the nail plate. It may be that the fungus is lying on the nail plate or below it, without infecting the nail. Proof of invasion of the fungous infection into the nail-plate itself is found only by means of the histologic examination. Furthermore, such examination seems even more important when one considers that frequently the results of cultures of fungi from scrapings of the nail are negative in spite of the fact that the examination in potassium hydroxide preparations shows that fungi are present. It may be that cultures from nail substances yield positive results less often than do cultures from the skin because of technical difficulties or contaminating overgrowth of non-pathogenic fungi or bacteria.

From the clinical point of view the histologic examination is an aid in answering the important question of whether the fungous-infected nail may be a focus for the recurrence of dermatophytosis, because it may make it possible to obtain the following information.

1. It may show the arrangement of the fungi in the nail plate and the effect of their invasion upon the nail structure.
2. It may show that the fungi are in the nail plate, thus confirming the positive microscopic finding, or it may show that the fungi are present only in the area surrounding the nail.
3. It may reveal fungi in the nail bed and in subungual cornified material.
4. It may show whether the clinical picture and the histologic finding are consistent.
5. It may be a guide to the selection of the treatment of the disease.

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In order to determine the extent of the agreement of the clinical picture of onychomycosis with the microscopic findings a study was made by Dostrovsky, Raubitschek and F. Sagher (1a, b.). Scrapings of over 2000 nails from the hands and feet of 118 patients with various dermatophytoses were examined microscopically under potassium hydroxide and cultures were made. The microscopic examinations of the nails revealed mycelia in 61 patients. Excluding the yeastlike fungi, growth of pathogenic fungi was noted in no more than 13 cases. Evulsion of nails was undertaken in several cases in which the culture yielded positive results and from several nails in which only the microscopic examinations showed the presence of fungi. Most of these patients suffered from recurrent eruptions of dermatophytosis and the local treatments of the nails were without

TABLE 1

Comparison of microscopic, culture, and histologic examinations of fungi in 55 nails

NUMBER OF PATIENTS	NUMBER OF NAILS	MICROSCOPIC EXAMINATION IN POTASSIUM HYDROXIDE; NO. OF NAILS WITH POSITIVE RESULTS	CULTURE RESULTS	HISTOLOGIC EXAMINATION; NO. OF NAILS WITH POSITIVE RESULTS
1	4	3	T. violaceum	3
4	19	19	T. purpureum (skin)	17
2	5	5	T. purpureum (nail)	5
3	14	11	Negative	3
3	12	3	Negative	4
			Non-pathogenic growth	Hyphae on the nail plate
1	1	0	Negative	Negative
Total 14	55	41	7	32

success. The suspicion arose that these diseased nails were foci which caused the recurrent skin eruptions.

MATERIAL

Fifty-five nails of 14 patients were examined histologically. Fifty-one nails were examined after evulsion, the four remaining nails were cut after a three weeks growth, and only the parings were examined.

In performing evulsion, the nails were cut in the center longitudinally. For histologic examination one-half of the nail was sectioned lengthwise and the other transversely. The transverse sections were cut in the middle or in the diseased part. This procedure makes possible the study of the arrangement of the fungi in the nail plate and also a survey of the greater part of the nail, a procedure which is essential because the entire nail is not always infected.

TECHNIQUE

The technical difficulties encountered have been one of the main reasons why the histologic examination of fungous-infected nails has been neglected up to the

present time. A method of procedure was described by MacLeod and Muende (2). They used Heller's (3) method, with fixation of the nails during several days in Mueller's solution,[1] prolonged washing, and decalcification from four to six days in one part of nitric acid and three parts of water, followed by embedding in celloidin. It is interesting to note, that this routine was suggested by MacLeod and Muende for the study of the nails in general, but was not mentioned in the chapter on onychomycosis where only examination in potassium hydroxide was recommended. Most dermatologists who have studied diseases of the nails have made use of decalcification or potassium hydroxide to bring about the separation of the cells (3, 4).

In the present investigation various methods and stains were tried on one nail in order to ascertain the simplest procedure. At first the nails were decalcified according to Heller's (3) method and embedded in celloidin or paraffin. Additional experiments, however, showed that the nail proved to be sufficiently prepared to be cut without the decalcification of frozen sections if it was soaked for several days in formalin and washed for twenty-four hours in running water. This method made the examinations easier and all of the nails were prepared in this manner.

STAINING METHODS

In order to find the best stain to use to detect fungi in the nails the following were tried: Hematoxylin-eosin, polychrome methylene blue, toluidine blue, van Gieson's, Gram's, Weigert's and Giemsa's stain. The best demonstration of fungi occurred with the hematoxylin-eosin stain, as follows: The sections were immersed in Ehrlich's acid hematoxylin for from eight to fifteen minutes and decolorized in 1 per cent hydrochloric acid diluted with 70 per cent alcohol. After staining for five minutes in 1 per cent eosin the sections were put into 70 per cent, 96 per cent and absolute alcohol, and finally mounted in Canada balsam. Each section was handled individually.

The mycelia with their structures were best shown by this staining method. Where Mayer's acid alum hematoxylin was used, part of the hyphae remained unstained. The cells of the malpighian layer as well as of the nail plate and the subungual keratosis became so dark that the fungi in these layers were not recognizable. Staining with hematoxylin Ehrlich made possible the examination of the nail plate and the underlying layers in spite of the different affinity of these layers for dyes, the color of the nail plate varying from pinkish to light purple and the underlying structures being dark purple. The spores and transverse sections of hyphae resembled small bubbles, of which part were stained dark red, while others appeared as light spots with the membrane stained but without darkened centers.

Other sections were left overnight in water after being stained with hematoxylin and were put into eosin the next day. In general, by means of this process the nail substance was lighter in color, the mycelia were stained well, their de-

[1] Potassium Bichromate 2.0–2.5, Sodium Sulfate 1.0, Distilled water 100.0.

tails became readily visible, and these sections were suitable for special examinations under the high power using oil-immersion lens. But part of the mycelia remained unstained. It was questionable whether the stain was lost overnight or whether the mycelia resisted the color, since some mycelia have been found unaffected by any staining method.

The modification of the Harris alum hematoxylin method was valuable, because it made possible the study of all the layers of the section of the nail (4). The nail plate appeared blue and the underlying epithelium yellow, and the fungi were clearly visible. Although the technique is more complicated, the use of a combination of Harris alum hematoxylin with Gram's stain has the advantage of making the search for fungi easier for the inexperienced investigator.

By staining with polychrome methylene blue the fungi can be made to appear almost unstained in the blue nail plate, where they are thus visible because of their light-refracting properties. The toluidine blue solution stained the fungi poorly and the color disappeared within one week.

With the use of van Gieson's stain the nail substance and the hyphae became greenish yellow. The central portion of the mycelia appeared somewhat darker green, sometimes even brown, with a pinkish hue; their protoplasm was lighter than the surrounding nail substance; and the subungual keratosis appeared brown. Examined with low power magnification the fungi were not easily recognizable because of their similarity to artefacts and splits in the nail, but upon examination with high power magnification the characteristics of the fungi and their segmentation were readily discernible. Even after two years the fungi remained stained, although their pinkish shade disappeared.

The remaining stains which were used may be summarized briefly. Gram's stain turned the nail to bluish purple; the fungi were difficult to distinguish and the mycelia were stained irregularly. After use of Weigert's elastic tissue stain the fungi remained unstained but they refracted light. Giemsa's stain made the nail plate appear blue, the epithelium underneath pink. The fungi were clearly discernible, their centers being dark and their peripheries light.

REPORT OF CASES

Case 1. Nine years before the first examination, a man, aged 19, observed changes in the appearance of his finger nails a short time after he had had tinea capitis. The microscopic examination of scrapings from these nails in potassium hydroxide preparations showed hyphae with segmentations at irregular distances. The segments were elongated and rectangular. The culture from these nails yielded a growth of Trichophyton violaceum. (Fig. 1a, b, c.)

After evulsion the nails were prepared for histologic examination by the afore-mentioned method. Upon examination the nail plate was found to be thick and wavy. Using van Gieson's stain, parts of the nail became yellow, others pink. The cells of the lower layers of the nail possessed nuclei and were darkly stained; the cells of the upper layers were indistinct and without nuclei. In the malpighian layer the cells and their membranes were very distinct and dark brown in color. In the upper part of the nail plate there were long mycelia with segmentation, yellow in color, which were scarcely distinguishable when they were examined under low power magnification, but were pronounced under high power magnification. In the lower part of the nail no mycelia were found. The fungus was embedded in the nail plate, without causing any reaction in the surrounding nail substance.

Parings of the first fingernail of the left hand were examined after the nail grew again following the second evulsion. There were no fungi present when microscopic and histologic examinations were made.

Case 2. A man, aged 50, suffered from onychomycosis for eight years. There was present subungual keratosis with a thickness of 3 mm. Fungi were found in the nails in microscopic examination under potassium hydroxide. The culture yielded a growth of T. purpureum Bang and the result of the intracutaneous trichophytin test was positive.

Histologic examination of both longitudinal and transverse sections of the nails and the underlying malpighian layer showed that the lowest layers of the nail plate, which were the

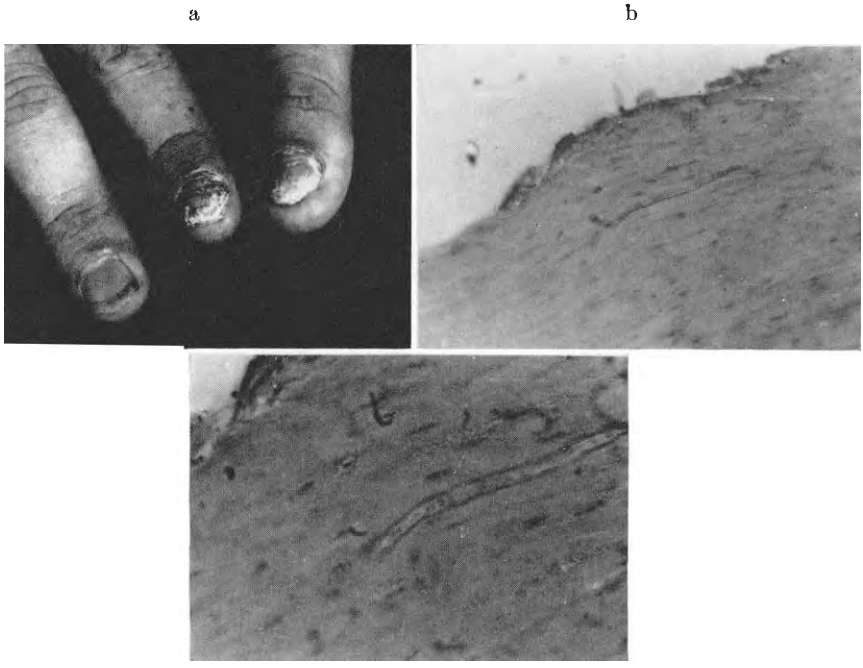

FIG. 1. CASE 1. ONYCHOMYCOSIS CAUSED BY TRICHOPHYTON VIOLACEUM

a. The nails are thickened, brittle, whitish and only partly transparent.

b. A long mycelium is seen in the superficial part of the nail plate. Frozen section, van Gieson, × 90.

c. The same segmented mycelium magnified 450 ×.

seat of the most outstanding changes, appeared wavy and sent deep processes into the nail bed; the normally straight line of the lower part of the nail plate resembled the epidermis and cutis with elongated papillary bodies and rete ridges, projections which were sometimes two or three times as long as the whole thickness of the nail plate; the fungi were present in the deepest layers of the nail plate and invaded these projections; and there were present thick and thin mycelia which were cut in longitudinal and transverse directions. The malpighian layer as well as the upper part of the nail was free.

Summary: The patient presented onychomycosis caused by T. purpureum Bang. The histologic examination of the affected nails revealed great changes in the structure of the nail plates. There were deep projections of nail substance penetrating the nail bed. Fungi were found throughout the nail in the deepest layers. No fungi were found in the malpighian layer.

Case 3. A woman, aged 30, had diseased toenails for ten years and noticed changes in the appearance of her fingernails for six years. Upon direct examination fungi were found in all twenty of the nails. Cultures from the skin and the nails produced T. purpureum Bang. The intracutaneous trichophytin test produced an immediate wheal which was 2 cm in diameter with pseudopodia surrounded by an erythema of 6 cm and appeared in 10 minutes. The results of the test were negative after twenty-four and forty-eight hours. Histologic examinations were made of the nails of both thumbs and both big toes as well as of 3 pieces of tissue from the nail beds of both of the hands and feet and the findings were almost the

a

b c

FIG. 2. ONYCHOMYCOSIS CAUSED BY TRICHOPHYTON PURPUREUM
a. Thickening of nails and subungual keratoses.
b. Longitudinal section. Note the mighty subungual keratosis. Frozen section, hematoxylin-eosin, × $8\frac{1}{4}$.
c. Transverse section. × 10.

same as those in case 2. (*Fig. 2a, b, c, and 3a, b, c.*) In addition there was a different arrangement of the fungi in the various layers of the nail plate: in the middle part the mycelia were short and often cut transversely; in the deeper layers they were long and septate, thin, and partly segmented. No fungi were found in the epithelium underneath the plate nor in the cutis of the nail bed.

Summary: The patient presented onychomycosis of the feet and hands caused by T. purpureum. The histologic examination revealed a great disturbance of layers of the nail plate. Fungi were present in the middle and lowest layers in different arrangements but were absent from the nail bed.

Case 4. A woman, aged 44, was being treated for an extensive eruption of tinea corporis. At the same time all of the nails of the toes were thickened, brittle, and whitish. Examination of portions of the skin and nails in potassium hydroxide revealed mycelia. A culture

from scrapings of the skin revealed the presence of T. purpureum, but the result of a culture from the nails was negative. The result of the intracutaneous trichophytin test was negative.

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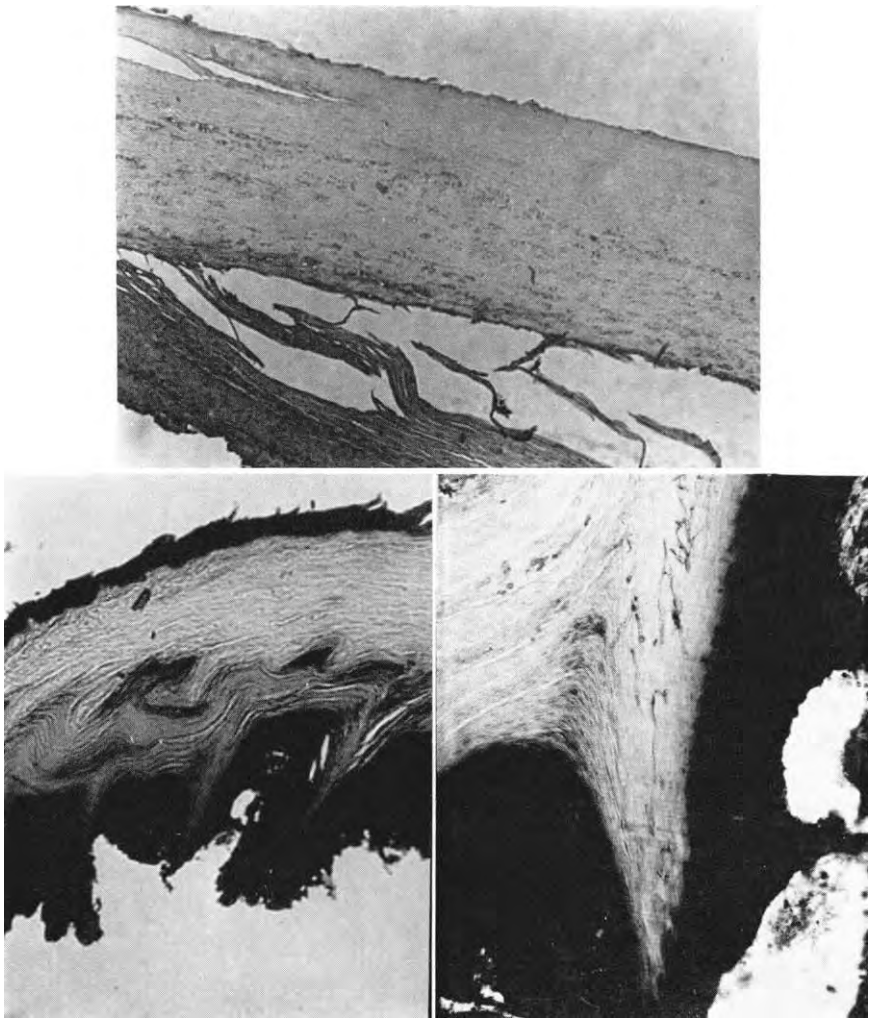

b c

FIG. 3. ONYCHOMYCOSIS CAUSED BY TRICHOPHYTON PURPUREUM

a. Fungi are present only in the lower part of the nail plate. Frozen section, hematoxylin-eosin, × 75.

b. In long-standing infections the lower part of the plate represents an irregular instead of a straight line and sends deep processes into the underlying tissue. Frozen section, hematoxylin-eosin, × 48.

c. Mycelia can be followed in these processes. × 225.

The histologic examination was made of the third and second nails of the right foot and the first and third nails of the left foot. Fungi were found in the third nail of the left foot and the second nail of the right foot. The latter, especially the middle part of the nail

plate, was filled with mycelia whose number decreased towards the lower layers. They appeared sporelike but were found to be mycelia cut in their transverse directions, a fact which could be established by following the continuations of the sporelike elements into the nail structure. The mycelia were twisted together in screwlike formations. In several places they were thinned out; in others they appeared puffed up. In the third nail of the left foot the fungi were found only in the lower quarter of the nail plate. Branched mycelia were present in several places. The presence of numerous collections of bacteria and amorphous masses in the malpighian layer disclosed the reason why cultures from the nails are so often overgrown by bacteria. Many spores were present in the subungual horny masses.

Case 5. A woman, aged 21, had tinea corporis for five years and changes in the formation of all the toenails. The result of the examination for fungi of the skin and nails was positive. The culture from material of the skin yielded a growth of T. purpureum; the nails yielded none. The intracutaneous trichophytin test produced an immediate wheal with pseudopodia and surrounding erythema. After forty-eight hours the results of the test became negative.

Histologic examinations of the nails showed that the mycelia were much shorter in the transverse than in the longitudinal sections and appeared to be cut transversely; the central portion of the nail were more diseased than the lateral parts; the fungi were present in one nail mainly at the nail root, in others only in the distal part. The fungi were found sometimes in the whole nail, in others only parts of the nail, but were almost always only in the deepest layers of the nail plate. The other features disclosed by histologic examination were the same as in case 2.

Case 6. A man, aged 38, had tinea corporis for eight years and onychomycosis on several fingernails for the last two years. The nails were raised by subungual horny masses which were a greyish black color. The direct examination of the skin and nails showed hyphae. The culture from material of the skin yielded a growth of T. purpureum, but none appeared in the culture from the nails. The result of the intracutaneous trichophytin test was negative.

Four nails and three pieces of tissue from the nail bed were examined histologically. Similar findings were observed in the longitudinal and transverse sections. Fungi were present in all of the nail plates, but in smaller number at their distal portions. The upper layers were free of fungi, the hyphae had not entered into the tissue below the nail plate. In one place a mycelium could be traced down to the epithelium where it turned upwards without entering this layer. The hyphae seemed to be under pressure and were screwlike in shape. Part of them showed chains of spores; others were almost unsegmented. The three pieces of tissue from the nail beds were embedded in paraffin and stained by the hematoxylin-eosin, van Gieson's and Gram's methods. The epidermis was only partly present in these sections since the greater part remained on the nail plates. The tissue was rich in blood vessels, chiefly large capillaries but also single veins; the connective tissue bundles were entwined and there were numerous cells. Several small cell infiltrations were seen around the vessels. Most of the cells possessed elongated nuclei similar to fibroblasts. There were many nerves cut in various directions. No fungi were found in the nail bed.

Case 7. A woman, aged 21, had an eczematous eruption on the first finger of her left hand for three years and a scaly lesion on her buttocks for one year. The nail of the finger was markedly abnormal and there was a thick subungual horny mass. Mycelia were found in a specimen from the dermatitis on the buttock as well as from the fingernail and eight toenails. T. purpureum was grown in a culture from the material of the skin.

The histologic examination of the nail plate showed the same features as in the preceding case: many chains of hyphae were present in the subungual horny masses, especially in the

part adjacent to the nail; in the deeper parts of the subungual horny substance fungi were not found; and there were also amorphous masses and many bacteria. The nail showed several deep splits into which hyphae were entering.

Case 8. A woman, aged 35, noticed changes in the appearance of her fingernails for one year. Mycelia were found in potassium hydroxide preparations made from the nail; the culture yielded no growth of pathogenic fungi. The nails were so brittle and broken, that it was impossible to obtain complete longitudinal and transverse sections for histologic examination. Mycelia were found, however, only in the lowest layers of the nail. They were long, with many segmentations in which a deeper colored formation resembling a nucleus was present. The findings were quite different in the layer above. This was filled with round and oval sporelike structures, (*Fig. 4a, b.*) of which part of the centers were dark stained and nucleus-like, while others were light spots of the same size without any cellular elements. Around several of these spores were bright halos which sent branches into the surrounding area. The spores had a destructive effect upon the nails, breaking them apart and giving them their characteristic appearance.

Case 9. A man, aged 40, had hyperkeratotic lesions on his feet and thickening of his toenails for ten years and thickening of his fingernails for one and one-half years. Direct examination showed short mycelia and yeastlike cells in the tissue; the culture yielded no growth of pathogenic fungi. Nine nails which had been removed were examined histologically. Since hyphae and spores were found only in the uppermost layer of the first toenail of the left foot, it seemed that the fungus entered the nail plate from the top. No fungi could be detected in tissue removed from the nail beds of three different toes.

Case 10. A woman, aged 31, had paronychia on several fingers with occasional purulent exudations for seven years. Hyphae were found by direct examinations of material from the nails; the culture yielded no growth of pathogenic fungi; and the result of the trichophytin test was negative.

Inside of the nails no fungi were apparent in the histologic examination, but fungi were found lying on top of the nails. These fungi had a different appearance from those within the nail substance itself (*Fig. 5a, b.*). Dense masses of hyphae were seen in one place upon examination with low power magnification, and dry high power magnification disclosed segmented mycelia with masses of spores, resembling slide cultures of fungi. These fungi lying on the nail plate branched out into the surrounding area, where no nail substance was present, but no hyphae were seen entering the nail substance. There were differences between the fungi in the nail substance and those lying on top of the nail plate: the first were always cut in short sections so that only short hyphae or sporelike structures, which were transverse sections of mycelia, could be found; the second could be traced for their entire length and masses of adherent spores were visible.

Case 11. A man, aged 30, suffered from recurrent eczematous eruptions of the hands and of different parts of the body for eight years. The nails of the hands and feet appeared abnormal for the last four years. Material from the nails examined under potassium hydroxide showed sporelike bodies, and the result of the intracutaneous trichophytin test was negative.

When 5 fingernails and 6 toenails were examined histologically, fungi were absent from the nail plates, but networks of mycelia were found lying on top of the first and second nails of the right foot. Their appearance was the same as the appearance of those in case 10.

Case 12. A man, aged 45, suffered from dyshidrotic eczema of the hands accompanied by abnormalities of the nails. Sporelike structures were found during direct examination

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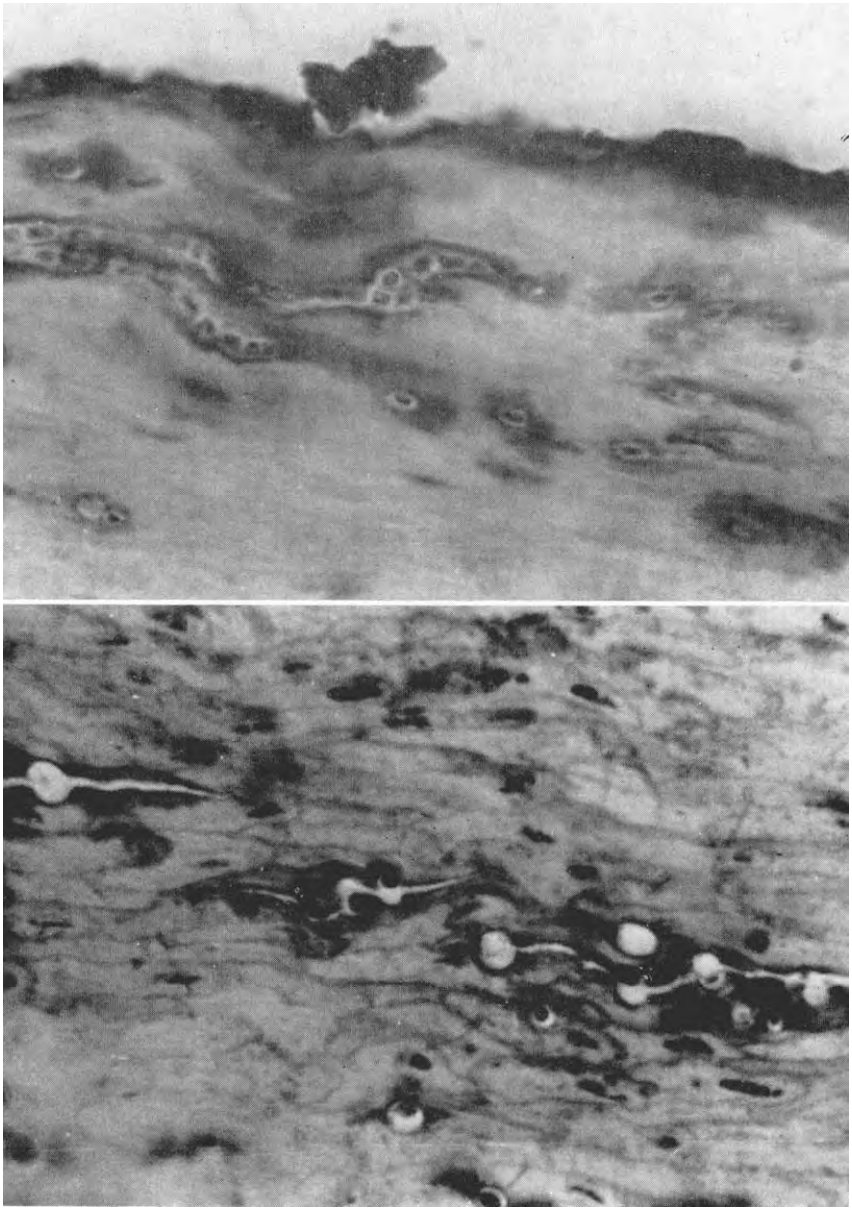

b

FIG. 4. CASE 8. ONYCHOMYCOSIS, CULTURE NEGATIVE FOR FUNGI

a. Segmented mycelia in the nail plate. The nail substance surrounding the hyphae is without substantial changes. Frozen section, hematoxylin-eosin, × 450.

b. Same case. Spores in the nail plate. Several spores are surrounded by a bright halo. Note the splits in their vicinity. Frozen section, Harris alum hematoxylin, × 450.

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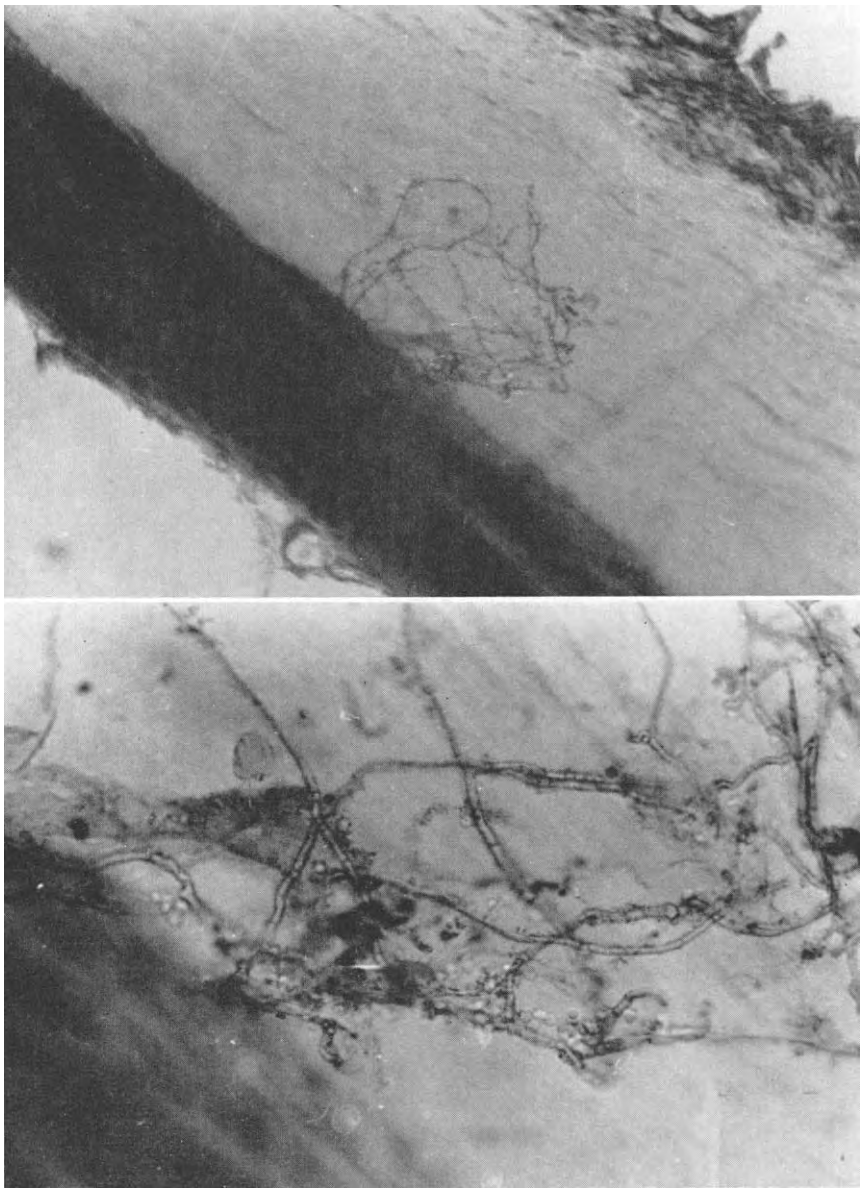

b

FIG. 5

a. Fungi lying on top of the nail plate. In potassium hydroxide preparations mycelia were found. The culture yielded no growth of pathogenic fungi. Histologically no fungi were found entering the nail plate. Frozen section, hematoxylin-eosin, × 100.

b. Same section magnified 450 ×. The aspect of the fungi is different from those in the nail plate. Note that the mycelia and spores are arranged in slide culture fashion.

of the nails; the culture from the nails produced no pathogenic fungi; the result of the trichophytin test was negative. The histologic findings were the same as those in case 10.

Case 13. A woman, aged 42, had noticed changes in her toenails for ten years and in the first, second and third fingernails of the right hand for one year. Spores were found on one fingernail and two toenails when they were examined directly; the culture yielded a growth of Candida tropicalis. Since the patient would not permit evulsion of the nails to be performed, nail parings were examined histologically but no fungi were found. It was impossible, therefore, to prove conclusively that fungi were present, because only particles of the nails were examined.

Case 14. A woman, aged 31, suffered from eczema and ulcers of the legs. All of the toenails showed changes similar to those produced by onychomycosis. Direct and histologic examinations of the nails and the culture made from them revealed no fungi.

COMMENT

The first description of fungi found in the nails were given by Mahon (5) in a report of cases of favus in 1829 and by Baum and Meissner (6) who in 1853 described changes in the appearance of the nails caused by molds. Pelizzari (7) reported 20 cases of onychomycosis in 1887 and stressed the fact that the disease occurs frequently. From this time on the literature concerning the fungous-infected nail became quite voluminous. Kittredge (8) reviewed 312 cases which had appeared in the English literature up to the years 1936. In spite of the fact that Becker (9), Montgomery and Casper (10), Sulzberger and Lewis (11), Taylor (12), Williams (13) and others stressed the importance of the fungous-infected nail as the focus of recurrent skin eruptions, systematic histologic examinations of the nail have not been performed. Even monographs and handbooks contain no mention of histologic findings in the examination of nails infected by fungi (2, 14, 15, 16, 17, 18, 19). Only Unna (20) gave an excellent description of the histologic examination of favus infected nails in 1896. Examinations of single nails were made by Virchow (21), Koebner (22), Pelizzari (7), Frei (23) and Fischer (24); these investigators did not make cultures. Baker (25) mentioned a histologic procedure for examinations for fungi of the nails, but apparently did not make, or did not report, any systematic use of it. Herrmann (26), however, made sections of the nail in order to determine the penetration of various agents. The only picture of fungi in the nail was found in the chapters which Heller (3) and Miescher (27) contributed to the Handbuch der Haut-und Geschlechtskrankheiten. The same photography was used by both authors, but neither mentioned the type of fungus which caused the disease of the nail.

Characteristics of Fungous Infection of the Nails as Shown by Histologic Examination

Trichophyton violaceum. It was surprising that only few mycelia and spores were found on histologic examination in spite of the fact that masses of fungi were seen on examination of scrapings in potassium hydroxide preparations. This differs from the findings of T. violaceum in hair, in which the roots of the

hairs are filled with spores arranged in chains and hyphae are seldom seen. The long mycelia in the nail were similar to those in the culture of T. violaceum. Since in most sections the mycelia were seen in the uppermost layers of the nail plate, it is suggestive that the nail was invaded by the fungus from that direction. Furthermore, the nail substance in the area surrounding the hyphae was almost entirely undisturbed. The localization of the fungus in the upper layers of the nail plate explained the tendency of onychomycosis caused by this parasite to heal spontaneously, as did several of the nails of the patient in case no. 1.

Experiments seemed to prove the theory of Krantz (28), Pinkus (29), Jessner (30), Silver and Chiego (31) and others who assumed that the nail plate with the malpighian layer grew distally from the roots. It appeared probable therefore that a fungus which found no favorable environment in the nail was expelled through this peripheral growth.

Trichophyton purpureum Bang. In two patients a growth of T. purpureum was obtained from material from the nails, and although the results of the cultures from the nails of four other patients were negative, T. purpureum was grown from specimens of the cutaneous lesions. The clinical picture and the observations made during the direct and histologic examinations of the nails were identical in these six patients.

According to the histologic findings, the growth of T. purpureum differed entirely from that of T. violaceum: T. purpureum was found chiefly in the deepest layers of the nail plate, sometimes in its middle part, but never in the superficial layers; the mycelia appeared larger, branched, and segmented; spores could not be detected with certainty in the nail plate, but were seen in masses in subungual horny substance; the structure of the nail plate was greatly disturbed; large splits were seen which were apparently caused by fungi invading the nail; the hyphae were found more frequently at the nail root than in the distal or lateral parts. In the central parts of the nail the hyphae were densely arranged in the longitudinal sections, but fungi could not always be found in the lateral parts of the transverse sections, although there was clinical evidence that the sides were abnormal.

In cases of long standing infections the lower part of the nail plate became wavy, like the epidermis and cutis. Deep processes which contained numerous fungi (Fig. 3 b, c.) extended from the nail plate into the underlying tissue. The fact that the hyphae appeared often in screw-shaped and snakelike formations, thick and swollen in some parts and thinned out in others, especially in the parts of the nail in which no splits had formed, indicated that the fungi appeared to live under unfavorable conditions in the nail before these changes became pronounced.

In the nine specimens, taken from the nail beds of three patients, no fungi could be detected; but where subungual hyperkeratoses occurred masses of spores and only occasionally mycelia were found.

Direction of growth

In the longitudinal sections, the mycelia appeared as long, branched strands whereas in most transverse sections they were short or sporelike in appearance.

From this observation it may be concluded that this fungus grew lengthwise in the nail plate.

Invasion of the Nail Infection.

Inasmuch as the fungi were observed only in the deepest and middle layers of the nail, and were located in its medial portion and at the nail root as well as at its distal part, it is possible that the fungus invaded the nail plate from its base at the distal part of the free edge or from the region of the lunula and grew toward the nail root. The lateral parts of the nail seemed only occasionally to be the point of entry of the infection. The T. purpureum never seemed to enter the nail from the superficial layers as T. violaceum did. It was also noticeable that, when it entered from the hyponychium T. purpureum appeared to proceed centripetally, growing in the direction opposite to that of the nail growth, but when the fungus entered from the region of the lunula it appeared to grow in the same direction as the nail grew.

The clinical picture did not always conform to the histologic findings; clinically nails infected by T. purpureum are altered throughout their entire structure in most cases (32), but in the histologic sections fungi were present only in parts of the nails.

Histologic Examinations of Nails with Positive Microscopic Findings in Potassium Hydroxide Preparations, but without Pathogenic Growth in Culture

1. Undetermined fungi in the nail plate. Striking changes were seen in the nails of case 8. In addition to the long and short mycelia which were present in the entire nail plate, large spores with a distinct surrounding halo were noticed; splits developed from these spores (Fig. 4 a, b.). The active breaking apart of the nail plate was clearly visible. Since the splits were only around the spores, whereas the nail substance remained almost unchanged in the vicinity of the hyphae, it seemed that the spores were the force which caused the nails to break apart.

2. Undetermined fungi on or under the nail plate. In four nails of three patients fungi were found lying on the nail plate without entering it (Fig. 5 a, b). The culture gave negative results or a growth of non-pathogenic fungi. It is apparent that the parasites could have a pathogenic effect without invading the nail plate by entering the space between the nail and the hyponychium and so raising the nail out of its bed (33). The possibilities exist also that these types of fungi grew on the surface of nails which were altered by other disorders. It is also conceivable that the fungi contaminated and grew during the preparation of the material for examination. Since these fungi were found in only four nails, whereas all nails were prepared in the same way, the last-named possibility seems improbable.

A number of authors have reported cases of onychomycosis from which non-pathogenic fungi were cultured and believed then to be the cause of the abnormalities of the nails (33, 34 a, b, c, 35 a, b, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 a, b, c,

46, 47). The demonstration, by histologic examination, of these fungi within the nail plate would support the idea that generally non-pathogenic fungi could be pathogenic for nails only.

INFERENCES AS TO THERAPY

Perhaps some inferences as to therapy may be drawn from these histologic examinations which show that different fungi can have different centers of localization, paths of invasion, and arrangement in the nails. In treating cases of infection with T. purpureum, in which the fungus was observed only in the deepest layers of the nail and in the subungual horny material without entering the tissue of the nail bed, a method devised by Kile and Welsh (48) and Epstein (49) of using a drill to shave off the diseased parts of the nail sometimes should give satisfactory results. But in cases of long-standing infection, in which the nail sends deep horny processes into the layers of the nail bed, this type of local therapy is too painful, because it is necessary to drill into the nail bed with its many nerve endings in order to reach and destroy all of the processes of the nail plate. Experience proved that even evulsion of the nails followed by curettage of the nail bed was not always a satisfactory treatment in order to remove all of the processes. However, Taylor's (50) electrosurgical destruction of the nail root which prevents regrowth of the nail is advisable only when the other procedures prove unsuccessful.

On the other hand, infections by T. violaceum, a fungus which is found chiefly in the superficial layers of the nail, should respond more readily to local therapy, making surgical treatment unnecessary.

Similar conclusions are possible after histologic investigations of other fungi. Thus, understanding the significance of the histologic picture may help to achieve the maximum therapeutic effect with a minimum of destructive or radical measures. Further investigations will be necessary to prove whether nail parings alone could give sufficient information for therapeutic inference.

SUMMARY

The systematic histologic examination is a diagnostic procedure which has not been generally employed in studying nails infected by fungi.

Fifty-five nails of 14 patients, therefore, were examined histologically. By this procedure different centers of localization, different arrangement and different invasion points of Trichophyton purpureum Bang and Trichophyton violaceum in the nails could be distinguished. It was found that these types damaged the nail in different manner and degree.

Fungi, observed during the ordinary potassium hydroxide examination of 4 nails were found by histologic examination to be lying on top of the nail plate without entering it, suggesting the possibility that the presence of fungi in potassium hydroxide examinations is not always sufficient for establishing the diagnosis of onychomycosis.

On the other hand, the histologic examination could prove a presence of "non-pathogenic fungi" in the nail plate, causing onychomycosis, and indicating

the possibility that supposedly non-pathogenic fungi may become pathogenic for nails only.

Histologic examinations were made also of tissue from the nail bed after evulsion of infected nails. It was found that the nail beds were not infected by fungi but that masses of spores and, only occasionally, mycelia were found in subungual horny masses.

The observations made during histologic examinations will perhaps prove helpful in indicating suitable treatment for different types of fungous infections of the nail.

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REFERENCES

1a. Dostrovsky, A., Raubitschek, F., and Sagher, F.: The role of the nail in dermatophytosis, Harefuah **33:** 30 (July 15) 1947.

1b. Sagher, F.: The laboratory aspect of fungous diseases of the skin and hair, Acta med. orient. **6:** 68 (March) 1947.

2. MacLeod, J. M. H., and Muende, I.: Practical Handbook of the Pathology of the Skin, ed. 3, New York, Paul B. Hoeber, Inc., 1946.

3. Heller, J.: Die Krankheiten der Naegel, in Jadassohn, J.: Handbuch der Haut-und Geschlechtskrankheiten, Berlin, Julius Springer, 1927, vol. 13, pt. 2.

4. Cowdry, E. V.: Microscopic Technique in Biology and Medicine, Baltimore, Williams & Wilkins Co., 1943.

5. Mahon, P. A., cited by Heller (3), p. 183.

6. Meissner, G., cited by Heller (3), p. 173.

7. Pelizzari, C.: Ueber das Trichophyton Tonsurans, Monatsh. f. prakt. Dermat. **6:** 1049 (Dec. 1) 1887.

8. Kittredge, H. E.: Ringworm of nails; consideration of its incidence, anatomic distribution and etiology, with suggestions as to probable modus operandi in infection and extension based upon review of 312 reported cases, and upon clinical observation, Urol. & Cutan. Rev. **38:** 875 (Dec.) 1934.

9. Becker, F.: Role of onychomycosis in recurrent dermatomycoses, Kentucky M. J. **40:** 266 (July) 1942.

10. Montgomery, R. M., and Casper, E. A.: Cutaneous manifestations of fungi causing dermatophytosis and onychomycosis and their treatment, J. A. M. A. **128:** 77 (May 12) 1945.

11. Sulzberger, Marion B., and Lewis, G. M.: Leukonychia trichophytica; report of case, M. Rec. **146:** 305 (Oct. 6) 1937.

12. Taylor, K. P. A.: Chronic onychomycosis, J. A. M. A. **130:** 107 (Jan. 12) 1946.

13. Williams, C. M.: Tinea of the nails as a source of reinfection in tinea of the feet; preliminary report, Arch. Dermat. & Syph. **18:** 730 (Nov.) 1928.

14. Brumpt, E.: Précis de parasitologie, ed. 5, Paris, Masson et Cie, 1936, vol. 2.

15. Dodge, C. W.: Medical Mycology; Fungous Diseases of Men and Other Mammals, St. Louis, C. V. Mosby Co., 1935.

16. Milian, G.: Les maladies des ongles., in Darier, J., and others: Nouvelle pratique dermatologique, Paris, Masson et Cie, 1936, vol. 7, p. 275.

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[2] Dr. L. Doljanski was killed on April 13, 1948 in Jerusalem.

17. Ormsby, O. S., and Montgomery, H.: Diseases of the Skin, ed. 7, Philadelphia, Lea and Febiger, 1948., p. 1407.
18. Sabouraud, R.: Maladies du cuir chevelu. III. Les Maladies cryptogamiques. Les Teignes, Paris, Masson et Cie, 1910.
19. Sutton, R. L., and Sutton, R. L., Jr.: Diseases of the Skin, ed. 10, St. Louis, C. V. Mosby Co., 1939, p. 1419.
20. Unna, P. G.: The Histopathology of the Diseases of the Skin, translated from the German with the assistance of the author by Norman Walker, Edinburgh, William F. Clay, 1896, p. 342.
21. Virchow, R., cited by Heller (3), p. 173.
22. Köbner, H., cited by Heller (3), p. 173.
23. Frei, W.: Eine Soormykose der Nägel bei einer Salvarsan-Dermatitis, Arch. f. Dermat. u. Syph. **129:** 404, 1921.
24. Fischer, W.: Mikroskopische mit Boraxmethylenblau gefärbte Schnitte durch einen Trichophytienagel, Zentralbl. f. Haut- u. Geschlechtskr. **12:** 436, 1924.
25. Baker, R. D.: The Histopathology of the Mycoses, Washington, Army Institute of Pathology, 1945, p. 59.
26. Herrmann, Franz: Personal communication to the author.
27. Miescher, G.: Trichophytien und Epidermophytien, in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten, Berlin, Julius Springer, 1928, vol. 11, p. 378.
28. Krantz, W.: Beitrag zur Anatomie des Nagels, Dermat. Ztschr. **64:** 239 (Aug.) 1932.
29. Pinkus, F.: Die normale Anatomie der Haut, in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten, Berlin, Julius Springer, 1927, vol. 1, pt. 1, p. 267.
30. Jessner, M.: Über eine neue Form von Nagelmykosen (Leukonychia Trichophytica) Arch. f. Dermat. u. Syph. **141:** 1, 1922.
31. Silver, H., and Chiego, B.: Nails and nail changes. VI. Modern concepts of anatomy and biochemistry of the nails, J. Invest. Dermat. **3:** 133 (April) 1940.
32. Taylor, K. P. A.: Dermatophytosis and onychomycosis, J. A. M. A. **128:** 750 (July 7) 1945.
33. Young, W. J.: Pigmented mycotic growth beneath the nail, Arch. Dermat. & Syph. **30:** 186 (Aug.) 1934.
34a. Bereston, E. S., and Waring, W. S.: Aspergillus infection of the nails, Arch. Dermat. & Syph. **54:** 552 (Nov.) 1946.
34b. Bereston, E. S., and Keil, H.: Onychomycosis due to aspergillus flavus. Arch. Dermat. & Syph. **44:** 420 (Sept.) 1941.
34c. Bereston, E. S., and Waring, W. S.: Onychomycosis and dermatomycosis caused by trichophyton rubrum and aspergillus nidulans, Arch. Dermat. & Syph. **52:** 162 (Sept.) 1945.
35a. Cochet, G.: Sur un nouveau champignon arthrosporé (Arthrographis langeroni n.g. n. sp.) agent pathogène d'une onychomycose humaine, Ann. de parasitol. **17:** 97 (March 1) 1939.
35b. Cochet, G.: Propriétés physiologiques d'Arthrographis langeroni Cochet, 1939, agent pathogène d'une onychomycose humaine, Ann. de parasitol. **19:** 157, 1942–1943.
36. de Mello, I. F., and Fernandes, L. A.: Onychomycose du medius et annulaire dûe à une levure du genre Torulopsis Berlese 1894, Mycopathologia **2:** 124, 1939.
37. Du Bois, C.: Onychomycoses non teigneuses, Dermatologica **86:** 57 (Aug.–Sept.) 1942.
38. Jessner, M., and Kleiner, S.: Über das Vorkommen von Sprosspilzen an normalen Nägeln und ihre Pathogenität, Arch. f. Dermat. u. Syph. **149:** 363, 1925.
39. Karrenberg, C. L.: Nagelepidermophytie mit positiver Kultur, Arch. f. Dermat. u. Syph. **158:** 431, 1929.
40. Kittredge, H. E.: Onychomycosis universalis trichophytina et epidermophyta; report of the seventh case thus far recorded in English, Arch. Dermat. & Syph. **34:** 398 (Sept.) 1936.

41. LAMPE, P. H. J.: Mycosis of Nail Bed Caused by Mycocandida onychophila, Geneesk. tijdschr. v. Nederl.-Indië, **80:** 1065 (April 23) 1940.
42. NEGRONI, P.: El problema de las onixis micóticas no específicas, Rev. argent. dermatosif. **26:** 194, 1942.
43. PARDO-CASTELLO, V.: Diseases of the Nails, ed. 2, Springfield, Ill., Charles C. Thomas, 1941.
44. ROCKWOOD, E. M.: A Study of fungus-infected nails, Arch. Dermat. & Syph. **22:** 395 (Sept.) 1930.
45a. SARTORY, A., AND SARTORY, R.: Un cas d'onychomycose dû à l'aspergillus fumigatus Fresenius, Bull. Acad. de med., Paris, **129:** 482 (July 17) 1945.
45b. SARTORY, A., SARTORY, R., HUFSCHMITT, G., AND MEYER, J.: Un cas d'onychomycose provoquée par un Eurotium nouveau: Eurotium diplocyste, n. sp., Compt. rend. Soc. de biol. **104:** 881 (July 4) 1930.
45c. SARTORY, A., SARTORY, R., AND MEYER, J.: Contribution à l'étude des onychomycoses. Deux périsporiacées nouvelles susceptibles de provoquer des affections unguéales et dermiques; Scopulariopsis minimus, n. sp.; Eurotium diplocyste, n. sp., Strasbourg-méd. **90:** 597 (Oct. 5) 1930.
46. TARANTELLI, E.: Onychie und Paronychie durch Pilze vom Typus "Yeast-like." Ihre pathogenetische Bedeutung, Dermat. Wchnschr. **97:** 1311 (Sept. 9); 1346 (Sept. 16) 1933.
47. WEIDMAN, F. D.: Penicillium Brevicaule var. Hominis Saccardo, 1877, Brumpt and Langeron, 1910, in an American Case of Ringworm of the Toes, Arch. Dermat. & Syph. **2:** 703 (Dec.) 1920.
48. KILE, R. L., AND WELSH, A. L.: The use of a drill on diseased nails, Arch. Dermat. & Syph. **42:** 1123 (Dec.) 1940.
49. EPSTEIN, S.: Examination of nails for fungi, Arch. Dermat. & Syph. **51:** 209 (March) 1945.
50. TAYLOR, K. P. A.: Permanent extirpation of nails in chronic onychomycosis, Am. J. Surg. **27:** 145 (Jan.) 1935.

DISCUSSION

DR. TIBOR BENEDEK: I would like to know what clinical picture the nails showed, and how the preparations were made. Were they removed by surgical operation as a whole, or were the nails spontaneously separated from the nail bed, and only this material used for histological purposes? These points would clarify the discussion of this problem. Onychomycosis is called today almost any and every involvement of the nail organ. True onychomycosis is a rare disease. I refer to the well known statistics of Sabouraud, 1910, in which he presented only one single case of onychomycosis among over 500 cases of dermatomycosis. He even questioned whether this case was a primary onychomycosis or a secondary invasion of the nail material by the fungus.

During the recent war I observed over 300 cases of dermatomycosis. The nails of fingers and toes were thoroughly examined and we could not find a single case of onychomycosis. Much of the material published in the literature as onychomycosis refers for the most part to material off the matrix, and fungi may easily grow in the detritus material of the nail. To find fungi in sections or cultures is not at all difficult. I would ask the presenter therefore, what the clinical picture of the nail was, and how the material was secured.

DR. MARION B. SULZBERGER: I would like to report informally on some studies which have been under way in my department for several years and to discuss this paper for Dr. Franz Herrmann and Dr. S. Robert Narins who are unable to be here. Dr. Herrmann and Dr. Narins studied histologic sections of numerous nails infected with Trichophyton purpureum from 1943 to 1946 at the New York Skin and Cancer Unit. The purpose of these investigations was not the histologic study as such, as has been the case in Dr. Sagher's

excellent work, but was an attempt to determine to what extent, and by what means it might be possible to carry antifungal remedies to the infected sites within nails by application of suitable therapeutic materials to the dorsa of the nails. Drs. Herrmann and Narins at this time do not intend to report on their results regarding the implications for a possible therapeutic approach, but wish merely to demonstrate the close similarity of their findings with the pictures Dr. Sagher has shown.

Nails which had been evulsed in the course of treatment of Tr. purpureum infections were collected for their studies. Only fairly well intact nail-plates were used, and none were mutilated, abnormally thin or incomplete in their configuration. A dye, Alpharurin 2 G ("Patent Blue") was dissolved as a visible tracer-substance in the selected "penetrating" vehicle ("Intraderm"—Wallace Laboratories) which was tested as a potential carrier of a

FIG. 6. SECTION THROUGH NAIL

Mycelium (Trichophyton Purpureum) below, but not within the upper third. The dark shade of this upper zone corresponds with the blue stain from the solution which had been rubbed upon the dorsum of the nail.

fungicide. The liquid, containing 0.4% of the dye, was rubbed on to the dorsal surface of the extracted nail under comparable conditions, for three or five minutes. Contamination of the unexposed concavity of the nail from the outside was carefully avoided. The liquid was allowed to dry, and sections were made 30 minutes, 3 hours, and 24 hours after the applications. The sectioning was carried out with very sharp scalpel blades in transverse direction through the entire width of the nails, perpendicularly from the lower, untreated surface to the dorsal surface. The specimens were fixed on a plate of ground glass transilluminated from below. The operator observed his manipulation through a mounted magnifying lense. The sections were mounted and well cleared by means of a solution of Damar-resin, 1 part by weight, in xylene, 1 part by volume. The specimens remained well preserved under the cover slip.

The preparations showed the presence of mycelium of Tr. purpureum only in the lower half of the nail, above the nail-bed, (Fig. 6) but never in the uppermost, dense nail-plate. This is, therefore, in full conformity with the findings of Dr. Sagher. And even under the best conditions and with the most penetrating vehicles available these uppermost layers are

FIG. 7. MYCELIUM IN NAIL. HIGH POWER VIEW

the precise zone which, with the procedures described, showed impregnation with the dye from the previously applied material. The zone in which the micro-organisms are present, in fact the entire thickness of the nails (Fig. 7) was stained only when the plate had been

artificially and deliberately softened, and sometimes even macerated by alkaline keratolytics prior to the application of the colored solution. Application of an emulsion of calcium thioglycolate on the dorsal surface served this purpose best, though potassium hydroxide (10% in water) had a similar effect. Although Narins and Herrmann have utilized and investigated this deliberate softening principle in the treatment of mycotic nail infections for several years they refrain from further comment at this time, since the procedure is still too cumbersome for general use, and the results do not yet permit evaluation.

DR. SAGHER, F. I am grateful to Dr. Benedek for his comment, and wish to thank Dr. Sulzberger for his valuable addenda to the substance of this paper.

I have attempted only to demonstrate the value of examining fungous infections of the nails by histologic methods. Further investigations may show whether histologic examinations of nail parings will yield sufficient information to indicate the mode of infection of nails by different types of fungi and, possibly, to point the way toward more adequate therapy.